

EPA/OPP MICROBIOLOGY LABORATORY
ESC, Ft. Meade, MD

Standard Operating Procedure
for
Biosafety in the Laboratory

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1.0 SCOPE AND APPLICATION:

- 1.1 The protocol outlines the required safety measures for working with the microorganisms in the laboratory with specific procedures given for *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella choleraesuis*, and *Mycobacterium bovis* BCG (see ref. 15.1).

2.0 DEFINITIONS:

- 2.1 *Mycobacterium bovis* BCG is a live attenuated vaccine strain used to vaccinate humans against infection by *Mycobacterium tuberculosis*.
- 2.2 BSC = biological safety cabinet
- 2.3 PPE = personal protective equipment
- 2.4 CDC = Centers for Disease Control and Prevention
- 2.5 NIH = National Institutes of Health
- 2.6 Manipulation of cultures/infectious microorganisms = handling any open vessel or plate containing microorganism, conducting culture transfers, streaking plates, pipetting culture, harvesting culture, seeding carriers, dropping carriers into disinfectant/neutralizer/subculture media tubes, and reading results from tubes and plates (unless plates are closed and wrapped with parafilm)
- 2.7 "Appropriate" disinfectant = EPA-registered hospital disinfectant (efficacious against *S. aureus*, *P. aeruginosa*, and *S. choleraesuis*) or hospital disinfectant with tuberculocidal claims (efficacious against *S. aureus*, *P. aeruginosa*, *S. choleraesuis*, and *M. bovis* BCG)
- 2.8 Spill = A spill is defined as a *biohazardous material out of control*. The quantity of the biohazardous material spilled is not the sole determining factor in deciding whether or not an event is classified as a spill. Rather, the essential issue is whether the biological agent, the location, and the quantity collectively cause the situation to be beyond the control of the laboratory worker.

3.0 HEALTH AND SAFETY:

- 3.1 To protect the laboratory worker from possible infection by microorganisms, the health and safety guidelines provided in this protocol and in the Centers for Disease Control and Prevention/National Institutes of Health (CDC/NIH) "Biosafety in Microbiological and Biomedical Laboratories," 4th ed. (see ref. 15.4) manual must be followed. The manual is available in the laboratory. All laboratory personnel are required to read and familiarize themselves with the sections on Biosafety Levels 2 and 3.
- 3.2 Laboratory workers must familiarize themselves with the laboratory's biosafety spill clean-up procedures (see sections 10.4 through 10.8). Biosafety spill clean-up procedures are posted in the laboratories.
- 3.3 Laboratory workers are required to participate in the Agency's Occupational Medical Surveillance Program as established by EPA Order 1460.1. The laboratory director evaluates the duties and responsibilities of the team and identifies the employees that are subject to exposure to chemical and biological agents in the laboratory. The names are forwarded to the ESC Safety, Health and Environmental Program (SHEMP) manager who has responsibility for coordinating the medical monitoring program. The program is administered through the Department of Health and Human Services/U.S. Public Health Service.
- 3.4 Medical emergencies are handled according to procedures outlined in the ESC Occupant Emergency Plan (OEP). All emergencies are reported to the SHEMP manager. The laboratory director is responsible for documenting medical emergencies or accidents.
- 3.5 Spills and accidents are handled according to the practices outlined in this SOP, as well as procedures referenced in the OEP and the ESC Chemical Hygiene Plan (CHP). All spills and accidents are reported to the laboratory director and the SHEMP manager. The laboratory director is responsible for documenting spills and accidents.
- 3.6 In accordance with the CDC/NIH guidelines (see ref. 15.4), the laboratory director may restrict access to the laboratory as specified under "special practices".

4.0 CAUTIONS:

- 4.1 The CDC/NIH "Biosafety in Microbiological and Biomedical Laboratories," 4th ed. (see ref. 15.4) manual presents recommended guidelines for working with microorganisms assigned to Biosafety Levels 1 through 4. Although these guidelines are not currently legally enforceable guidelines, they are considered to be international standards of practice. Should an exposure event occur, the CDC/NIH guidelines could take on the force of law in that the laboratory management could be held legally responsible for not following accepted standards of practice (Dr. Robert McKinney, Director of the Division of Safety/NIH; personal communication to M. Cottrill, 7/10/98). Consequently, the laboratory will comply with the CDC/NIH guidelines.
- 4.2 Chapter 6, Subchapter 2, entitled "Hard Surface Carrier Test Methods", of AOAC International's Official Methods of Analysis (1995) cautions that *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella choleraesuis* are potentially pathogenic bacteria, and instructs the laboratory worker to follow Biosafety Level 2 practices when working with these microorganisms (see ref. 15.3). The EPA/OPP Microbiology Laboratory will comply with Biosafety Level 2 guidelines when handling *Staphylococcus aureus*, *Pseudomonas aeruginosa*, or *Salmonella choleraesuis*.
- 4.3 The CDC/NIH manual assigns propagation and manipulation of cultures of *Mycobacterium tuberculosis* and *Mycobacterium bovis* to Biosafety Level 3. The manual does not specifically address propagation and manipulation of cultures of *Mycobacterium bovis* BCG. NIH recommends that laboratories follow Biosafety Level 3 guidelines when handling *M. bovis* BCG (Dr. Robert McKinney, personal communication to M. Cottrill, 7/23/96). Those laboratories unable to meet the Biosafety Level 3 facility requirements may, at a minimum, handle cultures of *M. bovis* BCG in a Biosafety Level 2 facility while employing Biosafety Level 3 practices.

M. bovis BCG is as transmissible to humans via the aerosol route as *M. bovis*. If a laboratory worker seroconverts (i.e., has a positive tuberculosis skin test), it is impossible to determine with absolute certainty that the seroconversion was due to exposure to *M. bovis* BCG and not *M. tuberculosis*. Consequently, chest x-rays and drug therapy may become necessary to ensure that the worker does not have an active tuberculosis infection and is not infectious.

The EPA/OPP Microbiology Laboratory will comply with Biosafety Level 3 guidelines when handling *Mycobacterium bovis* BCG.

5.0 INTERFERENCES: None

6.0 PERSONNEL QUALIFICATIONS: Personnel are required to be knowledgeable of the procedures in this SOP. Documentation of training and familiarization with this SOP can be found in the training file for each employee. Each analyst will complete safety re-certification training on at least an annual basis.

7.0 SPECIAL APPARATUS AND MATERIALS:

7.1 Biological Safety Cabinet

7.2 Autoclave

7.3 Personal protective equipment (PPE) such as latex gloves, surgical masks, safety glasses, face shields, lab coats, and disposable laboratory garments.

7.4 Biosafety Spill Kit containing items such as autoclave bag, latex gloves, gloves for handling broken glass, dustpan/brush, shoe covers, disposable lab coat, paper towels, masks, disinfectant, safety glasses.

7.5 Appropriate signs to identify biohazardous materials and to limit access to laboratories.

8.0 INSTRUMENT OR METHOD CALIBRATION: None

9.0 SAMPLE HANDLING AND STORAGE: None

10.0 PROCEDURE AND ANALYSIS:

10.1 Biosafety Level 2

Biosafety Level 2 recommendations were designed for handling microorganisms which pose a moderate danger to humans. A recapitulation of the recommendations most pertinent to the EPA/OPP Microbiology Laboratory follows:

10.1.1 Follow Biosafety Level 2 practices when working with

Staphylococcus aureus, *Pseudomonas aeruginosa*, and *Salmonella choleraesuis*, and any other Biosafety Level 2 microorganism.

- 10.1.2 Limit access to the laboratory when manipulating infectious microorganisms (see section 2.6) by posting the magnetic "STOP/DO NOT ENTER" sign on the outside (i.e., side facing corridor) of the laboratory door. Only laboratory staff are authorized to enter the laboratory while the sign is posted.
- 10.1.3 Safety glasses must be worn while working in the laboratory. Safety glasses do not have to be worn while doing paperwork in the laboratory or when entering the laboratory solely to retrieve an item such as a document, *provided that no manipulation of cultures (see section 2.6) is in progress.*
- 10.1.4 Wear lab coats while working in the laboratory. Re-usable cotton lab coats or disposable lab coats are permissible. Lab coats do not have to be worn while doing paperwork in the laboratory or when entering the laboratory solely to retrieve an item such as a document, *provided that no manipulation of cultures (see section 2.6) is in progress.*
- 10.1.5 See section 10.7 for steps to take in the event of overt contamination of a cotton lab coat with infectious material.
- 10.1.6 Wear gloves when manipulating culture (see section 2.6) and when handling any vessel (e.g., test tube rack, test tube, plate, biohazard bag), closed or open, containing live organism.
- 10.1.7 Replace gloves immediately in the event of overt contamination with infectious material. Dispose of contaminated gloves in a biohazard bin.
- 10.1.8 Conduct all activities having the potential for creating aerosols (i.e., vortexing, sonicating, transferring cultures, examining/counting open agar plates) of *S. aureus*, *P. aeruginosa*, or *S. choleraesuis* in the BSC. Wear face protection (e.g., face mask) if, when working outside the hood, splashes or sprays are anticipated.

- 10.1.9 BSC surfaces must be disinfected prior to and after working with infectious material and after any spill of infectious material. Spray the surface of the BSC with the use dilution of an appropriate disinfectant. Allow the surface to remain wet for the label-specified contact time, and then dry the surface with paper towels.
- 10.1.10 Follow Biosafety Level 2 guidance when Gram staining *S. aureus*, *P. aeruginosa*, or *S. choleraesuis*. While Gram staining and viewing slides, wear gloves and a lab coat, and conduct any aerosol-generating steps in the BSC (e.g., smear preparation). Collect the rinsate and add household bleach full strength to the rinsate in order to obtain an approximate final concentration of 10% household bleach (see ref. 15.2, pg. 390) for a minimum of 10 minutes before disposal down the drain.
- 10.1.11 After microscopically viewing organisms, remove slides from the microscope stage and discard them in a biohazard bin. If it is necessary or desirable to keep a prepared slide, store it in a sealed petri dish or a microscope slide case to which a biohazard label has been affixed.
- 10.1.12 Clean and disinfect refrigerated recirculating waterbaths (i.e., chillers) as described in SOP QC-04-01 (see ref. 15.5).
- 10.1.13 If the sonicator has been used in a given week for product testing, disinfect the water in the sonicator bath, at the end of the testing week, by adding appropriate disinfectant to the water in the bath to achieve the disinfectant product's use dilution. Let the disinfectant remain in the sonicator bath for at least 10 minutes or the contact time stated on the disinfectant labeling. Once the contact time is achieved, discharge the treated water into the lab sink and dry the sonicator bath with paper towels.
- 10.1.14 After manipulating culture (see section 2.6), analysts must bag biohazardous waste and place it in a closed container (e.g., biohazard bin with lid).

- 10.1.15 Remove lab coats before going to non-laboratory areas such as the office areas, restrooms, cafeteria, library, etc.
- 10.1.16 Remove the "STOP/DO NOT ENTER" sign once work is complete.
- 10.1.17 Do not transport the live cultures outside of the microbiology laboratory wing.

10.2 Biosafety Level 3

Biosafety Level 3 recommendations were designed for handling microorganisms which may present a serious threat of disease to humans exposed to the agent by inhalation. A recapitulation of the recommendations most pertinent to the EPA/OPP Microbiology Laboratory follows:

- 10.2.1 All procedures involving *M. bovis* BCG must be performed in a Biosafety Level 3 laboratory (Rooms B202 and B207).
- 10.2.2 Follow Biosafety Level 3 practices when working with *M. bovis* BCG.
- 10.2.3 Limit access to the Biosafety Level 3 laboratory when manipulating infectious microorganisms (see section 2.6) by posting the magnetic "STOP/DO NOT ENTER" sign on the outside of the laboratory door leading from the corridor to the double-door access zone. The "STOP/DO NOT ENTER" sign will be posted immediately prior to manipulating cultures (see section 2.6). Only analysts associated with testing or manipulation of cultures are authorized to enter the Biosafety Level 3 laboratory while the sign is posted.
- 10.2.4 Safety glasses must be worn while working in the Biosafety Level 3 laboratory. Safety glasses do not have to be worn while doing paperwork in the laboratory or when entering the laboratory solely to retrieve an item such as a document, *provided that no manipulation of cultures (see section 2.6) is in progress.*

- 10.2.5 Wear disposable lab coats while working in the Biosafety Level 3 laboratory. White cloth lab coats may not be worn in the Biosafety Level 3 laboratory. Lab coats do not have to be worn while doing paperwork in the laboratory or when entering the laboratory solely to retrieve an item such as a document, *provided that no manipulation of cultures (see section 2.6) is in progress.*
- 10.2.6 Replace disposable lab coat immediately in the event of overt contamination with infectious material. Dispose of contaminated lab coat in a biohazard bin.
- 10.2.7 **Wear a double layer of gloves when manipulating culture** (see sections 2.6 and 10.2.9) . Prior to leaving the BSC to conduct other activities (e.g., open the incubator, record data, retrieve supplies, etc.), the outer pair of gloves must be discarded in the biohazard bin. Replace the outer gloves upon returning to the BSC.
- Wear at least a single pair of gloves when inspecting or moving any sealed vessel (e.g., tube, plate) containing *M. bovis* BCG including cultures in test tubes in racks stored in plastic bags. In addition, a single pair of gloves will be worn when preparing biohazardous waste for autoclaving.
- 10.2.8 Replace gloves immediately in the event of overt contamination with infectious material. Dispose of contaminated gloves in a biohazard bin.
- 10.2.9 All procedures requiring the manipulation (see section 2.6) of *M. bovis* BCG, not solely aerosol-generating procedures, must be conducted within a BSC. If a certain procedure involving manipulation of the organism is impossible or impractical to conduct within the BSC (e.g., reading the percent transmittance of the *M. bovis* BCG culture), NIOSH-certified TB respirators or face shields must be worn while working with the organism outside of the BSC. Transporting closed petri dishes (containing seeded carriers) to the incubator for drying and counting colonies on plates which are closed and wrapped with parafilm are not considered to be manipulation of culture.

Wearing TB respirators or face shields are not mandatory for these two activities.

- 10.2.10 Ink pens and clip boards used to record data while manipulating microorganisms (see section 2.6) will be identified for use in Biosafety Level 3 laboratories.
- 10.2.11 For confirmatory tuberculocidal testing, conduct all steps of the procedure in the BSC, with the exception of reading the % transmittance of the culture, drying the carriers, and counting colonies on plates which are closed and wrapped with parafilm.
- Specifically, conduct the following manipulations in the BSC: handling any open vessel or plate containing microorganism, conducting culture transfers, streaking plates, pipetting culture, harvesting culture, seeding carriers, dropping carriers into disinfectant/neutralizer/ subculture media tubes, and reading results from tubes and plates (unless plates are closed and wrapped with parafilm).
- 10.2.12 BSC surfaces must be disinfected prior to and after working with infectious material and after any spill of infectious material. Spray the surface of the BSC with the use dilution of an appropriate disinfectant. Allow the surface to remain wet for the label-specified contact time, and then dry the surface with paper towels.
- 10.2.13 Use Biosafety Level 3 guidance when conducting acid-fast staining of *M. bovis* BCG. While staining and viewing slides, wear gloves and a lab coat, and conduct any steps involving the manipulation of the organism in the BSC (e.g., smear preparation). Collect the rinsate and add household bleach full strength to the rinsate in order to obtain an approximate final concentration of 10% household bleach (see ref. 15.2, pg. 390) for a minimum of 10 minutes before disposal down the drain.
- 10.2.14 After microscopically viewing organisms, remove slides from the microscope stage and discard them in a biohazard bin. If

it is necessary to keep a prepared slide, store it in a sealed petri dish or a microscope slide case to which a biohazard label has been affixed.

- 10.2.15 Clean and disinfect refrigerated recirculating waterbaths (i.e., chillers) as described in SOP QC-04-01 (see ref. 15.5).
- 10.2.16 If the sonicator has been used in a given week for product testing, disinfect the water in the sonicator bath, at the end of the testing week, by adding appropriate disinfectant to the water in the bath to achieve the disinfectant product's use dilution. Let the disinfectant remain in the sonicator bath for at least 10 minutes or the contact time stated on the disinfectant labeling. Once the contact time is achieved, discharge the treated water into the lab sink and dry the sonicator bath with paper towels.
- 10.2.17 After manipulating culture (see section 2.6), analysts must bag biohazardous waste and place it in a closed container (e.g., biohazard bin with lid).
- 10.2.18 Remove and discard lab coats (in biohazard bin) before exiting the Biosafety Level 3 laboratory to enter the double-door access zone. No lab coats worn during manipulation (see section 2.6) of *M. bovis* BCG may be worn out of the Biosafety Level 3 laboratory.
- 10.2.19 Remove the "STOP/DO NOT ENTER" sign once work is complete.
- 10.2.20 When removing live *M. bovis* BCG cultures (i.e., agar plates, racks of tubes, biohazard bags containing biohazardous waste) from the immediate Biosafety Level 3 laboratory for incubation in other laboratories or for decontamination purposes, close the cultures (i.e., put agar plates and racks of tubes in plastic bags and tape shut) and place the closed cultures in durable, leak-proof containers (e.g., nalgene tub) for transport from the laboratory. Autoclave bags containing biohazardous waste should be taped shut prior to transport. **Do not transport the live *M. bovis* BCG cultures outside of the**

microbiology laboratory wing.

Remove and discard disposable lab coat and gloves prior to exiting the Biosafety Level 3 laboratory and replace them with a clean (previously unexposed to *M. bovis* BCG) coat and a new set of gloves, to be worn during the transport process.

- 10.3 Prior to manipulating (see section 2.6) cultures of a microorganism other than *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella choleraesuis*, and *Mycobacterium bovis* BCG, the laboratory staff must first determine the biosafety level of that microorganism (see refs. 15.2 and 15.4). Biosafety level 3 microorganisms may only be manipulated in a biosafety level 3 laboratory. Biosafety level 2 microorganisms may be manipulated in either a biosafety level 2 or a biosafety level 3 laboratory.
- 10.4 Guidance for Spills of Biohazardous Material - Reporting Instructions
 - 10.4.1 Accidents are handled according to the practices outlined in this subpart, as well as procedures referenced in the OEP and the ESC Chemical Hygiene Plan. *All spills and accidents* are reported to the laboratory director and the SHEMP manager. The laboratory director is responsible for documenting spills and accidents.
- 10.5 Guidance for Spills of Biohazardous Organisms Requiring Biosafety Level 2 Containment
 - 10.5.1 Alert workers in the laboratory of the spill.
 - 10.5.2 In the event that human injury occurs as a result of a spill of biohazardous material (e.g., cut from broken glass), request assistance from co-worker and treat the injury prior to initiating cleanup procedures. Once the injury is treated, proceed with clothing decontamination and cleanup procedures.
 - 10.5.3 Remove contaminated clothing.
 - 10.5.3.1 If disposable lab coat becomes contaminated, discard it in the appropriate biohazard bin.

- 10.5.3.2 If cotton lab coat becomes contaminated, remove coat and place in autoclavable container.
- 10.5.3.3 Inspect street clothing/footwear for potential contamination. If contaminated, remove and place in autoclavable container. If liquid soaked through clothing/footwear to the skin, wash skin with soap and water. Have a co-worker retrieve the jumpsuit from the shower area of the restroom (D222 or D223) or something else that will suffice as temporary clothing. If no one is available to assist, remove contaminated footwear and as much clothing as possible, put on shoe covers (in biosafety spill kit), and retrieve the jumpsuit.
- 10.5.4 Retrieve biosafety spill kit and open it.
- 10.5.5 Put on protective equipment, including lab coat, gloves, safety glasses, shoe covers (if material spilled onto the floor), and mask (if material spilled outside of the BSC). All items are in the biosafety spill kit.
- 10.5.6 Assess spill to determine if there is broken glass. If so, recognize that special care must be taken to avoid injury during cleanup.
- 10.5.7 Unplug any contaminated small equipment (e.g., vortex, timer).
- 10.5.8 For horizontal surfaces, cover entire affected area, including broken glass, with paper towels in biosafety spill kit in order to contain aerosols. Do not use plastic-backed paper or absorbent wipers.
- 10.5.9 Saturate paper towels, starting with the edges of the spill and working with a 10% bleach solution (1:10 dilution of household bleach; see ref. 15.2, pg. 390), or other appropriate disinfectant. Let stand for 20 to 30 minutes.

- 10.5.10 For vertical surfaces, saturate area with disinfectant and let stand for 20 to 30 minutes. Wipe up area with absorbent wipers.
- 10.5.11 Pick up absorbent material and place in the autoclave bag found in the biosafety spill kit. If there is broken glass under the absorbent paper, use the gloves (biosafety spill kit) designed for handling glass to pick up the absorbent paper and the brush and scoop to pick up the glass and transport it to the autoclave bag. UNDER NO CIRCUMSTANCES SHOULD AN ANALYST ATTEMPT TO PICK UP BROKEN GLASS WITH A LATEX-GLOVED HAND!
- 10.5.12 Place any contaminated writing utensils in the autoclave bag.
- 10.5.13 Autoclave and dispose of autoclave bag.
- 10.5.14 Decontaminate gloves (used to handle glass), scoop, brush, and the lid/handle of the biosafety spill kit with disinfectant and return to spill kit.
- 10.5.15 Treat contaminated cotton lab coat and street clothing/footwear as indicated in sections 10.7 and 10.8, respectively.
- 10.5.16 Replenish biosafety spill kit supplies.
- 10.6 Guidance for Spills of Biohazardous Organisms Requiring Biosafety Level 3 Containment
 - 10.6.1 Spills *outside of the BSC* (risk of inhalation exposure):
 - 10.6.1.1 Worker should alert others in the laboratory of the spill and instruct all workers to remove lab coat and gloves, discard them in the biohazard bin, and to evacuate the laboratory immediately to the double-door access room.
 - 10.6.1.2 Close door to laboratory.

- 10.6.1.3 In the event that human injury occurs as a result of a spill of biohazardous material (e.g., cut from broken glass), treat the injury.
- 10.6.1.4 Remove contaminated street clothing/footwear basically as described in section 10.6.2.5. However, note that the inability to re-enter the laboratory (section 10.6.1.5) necessitates improvisation to avoid contaminating the hallway.
- 10.6.1.5 Workers must not re-enter the laboratory to decontaminate surfaces or clean up the spill for at least 30 minutes. During this time, the infectious bioaerosols will be removed from the laboratory by the facility's exhaust air ventilation system.
- 10.6.1.6 Retrieve biosafety spill kit from another laboratory and open it.
- 10.6.1.7 Put on protective equipment, including lab coat, gloves, safety glasses, mask, and shoe covers (if material spilled onto the floor). All items are in the biosafety spill kit.
- 10.6.1.8 After 30 minutes has passed, staff, dressed in protective equipment, may re-enter the laboratory and proceed with decontamination/clean up procedures (section 10.6.3).
- 10.6.2 Spills *inside of the BSC* (bioaerosols will be contained by the BSC's laminar air flow):
 - 10.6.2.1 Alert workers in the laboratory of the spill.
 - 10.6.2.2 Keep the BSC operational-do not turn off the blower.

- 10.6.2.3 In the event that human injury occurs as a result of a spill of biohazardous material (e.g., cut from broken glass), treat the injury.
- 10.6.2.4 If disposable lab coat becomes contaminated, discard it in the appropriate biohazard bin.
- 10.6.2.5 Inspect street clothing/footwear for potential contamination. If contaminated, remove and place in autoclavable container. If liquid soaked through clothing/footwear to the skin, wash skin with soap and water. Have a co-worker retrieve the jumpsuit from the shower area of the restroom (D222 or D223) or something else that will suffice as temporary clothing. If no one is available to assist, remove contaminated footwear and as much clothing as possible, put on shoe covers (in biosafety spill kit), and retrieve the jumpsuit.
- 10.6.2.6 Retrieve biosafety spill kit and open it.
- 10.6.2.7 Put on protective equipment, including lab coat, gloves, and safety glasses. All items are in the biosafety spill kit.
- 10.6.2.8 Proceed with decontamination/clean up procedures (section 10.6.3).
- 10.6.3 Assess spill to determine if there is broken glass. If so, recognize that special care must be taken to avoid injury during cleanup.
- 10.6.4 Unplug any contaminated small equipment (e.g., vortex, timer).
- 10.6.5 For horizontal surfaces, cover entire affected area, including broken glass, with paper towels in biosafety spill kit in order to contain aerosols. Do not use plastic-backed paper or absorbent wipers.

- 10.6.6 Saturate paper towels, starting with the edges of the spill and working with a 10% bleach solution (1:10 dilution of household bleach; see ref. 15.2, pg. 390), or other appropriate disinfectant. Let stand for 20 to 30 minutes.
- 10.6.7 For vertical surfaces, saturate area with disinfectant and let stand for 20 to 30 minutes. Wipe up area with absorbent wipers.
- 10.6.8 Pick up absorbent material and place in the autoclave bag found in the biosafety spill kit. If there is broken glass under the absorbent paper, use the gloves (biosafety spill kit) designed for handling glass to pick up the absorbent paper and the brush and scoop to pick up the glass and transport it to the autoclave bag. UNDER NO CIRCUMSTANCES SHOULD AN ANALYST ATTEMPT TO PICK UP BROKEN GLASS WITH A LATEX-GLOVED HAND!
- 10.6.9 Place any contaminated writing utensils in the autoclave bag.
- 10.6.10 Autoclave and dispose of autoclave bag.
- 10.6.11 Decontaminate gloves (used to handle glass), scoop, brush, and the lid/handle of the biosafety spill kit with disinfectant and return to spill kit.
- 10.6.12 Treat contaminated street clothing/footwear as indicated in section 10.8.
- 10.6.13 Replenish biosafety spill kit supplies.
- 10.7 Guidance for Spills of Biohazardous Material - Decontamination of Cotton Lab Coat
 - 10.7.1 Decontaminate cotton lab coat with an appropriate disinfectant or by autoclaving.
 - 10.7.1.1 If using disinfection as a means of decontamination, retrieve disinfectant. Treat area of contamination and surrounding area with

disinfectant for label-specified contact time.

10.7.1.2 It is less harmful to clothing to autoclave it in a tray than it is to bag it. Do not put water in the tray with the lab coat. Rather, put a second tray into the autoclave and add water to this tray.

10.7.2 After lab coat is decontaminated (by disinfection or autoclaving), immerse it in water containing detergent to aid physical removal of decontaminated biohazardous material.

10.7.3 Rinse lab coat and set aside to be sent out with the weekly lab coat laundry service.

10.8 Guidance for Spills of Biohazardous Material - Decontamination of Street Clothing/Footwear

10.8.1 Decontaminate clothing/footwear with an appropriate disinfectant or by autoclaving.

10.8.1.2 If using disinfection as a means of decontamination, retrieve disinfectant. Treat garment with disinfectant for label-specified contact time.

10.8.1.3 It is less harmful to clothing to autoclave it in a tray than it is to bag it. Do not put water in the tray with the lab coat. Rather, put a second tray into the autoclave and add water to this tray.

10.8.2 After clothing/footwear is decontaminated (by disinfection or autoclaving), immerse it in water containing detergent to aid physical removal of decontaminated biohazardous material.

10.8.3 Take clothing home and launder.

10.9 Several of the differences between the biosafety levels are highlighted in the table below (see ref. 15.4):

Biosafety Level	Agents	Practices	Safety Equipment	Facilities
1	Not known to consistently cause disease in healthy adults.	Standard Microbiological Practices.	None required.	Open bench top sink required.
2	Associated with human disease, hazard = percutaneous injury, ingestion, mucous membrane exposure	BSL-1 practice plus: *Limited access *Biohazard warning signs *"Sharps" precautions *Biosafety manual defining any needed waste decontamination or medical surveillance policies	Primary barriers= Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials; PPEs: laboratory coats; gloves; face protection as needed	BSL-1 plus: Autoclave available
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious or lethal consequences	BSL-2 practice plus: *Controlled access *Decontamination of all waste *Decontamination of lab clothing before laundering *Baseline serum	Primary barriers= Class I or II BSCs or other physical containment devices used for all open manipulations of agents; PPEs: protective lab clothing; gloves; respiratory protection as needed	BSL-2 plus: *Physical separation from access corridors *Self-closing, double door access *Exhausted air not recirculated *Negative airflow into laboratory

11.0 DATA ANALYSIS/CALCULATIONS: None

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT: None

13.0 QUALITY CONTROL:

13.1 The EPA/OPP MICROBIOLOGY LABORATORY conforms to 40CFR Part 160, Good Laboratory Practices. Appropriate quality control measures are integrated into each SOP.

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

- 14.1 Strict adherence to the biosafety practices is required. Nonconformance will result in notification, retraining, or disciplinary action of laboratory employees.

15.0 REFERENCES:

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- 15.3 Official Methods of Analysis. 1995. 16th Ed. AOAC INTERNATIONAL, Gaithersburg, MD.
- 15.4 Richmond, J.Y. and McKinney, R.W. eds. 1999. Biosafety in Microbiological and Biomedical Laboratories. HHS Publication No. (CDC) 93-8395. U . S . Government Printing Office, Washington, D.C.
- 15.5 Rindal, M. 2000. SOP QC-04-01 Cleaning and Disinfection of Recirculating Chillers and Enumeration of Heterotrophic Bacteria in Chiller Water.

16.0 FORMS AND DATA SHEETS: None

Standard Operating Procedure (SOP) Addendum Form

OPP Microbiology Laboratory

(Addendum: A-1)

SOP Name :	Biosafety in the Laboratory
SOP Number:	MB-01-02
Changes made by (name/date):	Michele Cottrill/3-14-01
Description of Change(s):	See attached sheet for change.
Justification(s) for the Change(s):	1. Limited dexterity when wearing a double layer of gloves.
	2. Alternate method of reducing microbial contamination of work surfaces outside of the BSC.
Approval: (Signature/Date)	
OPP Lab QA Officer	
OPP Lab Team Leader	

10.2.7 Wear gloves when handling any vessel (e.g., test tube rack, test tube, plate, biohazard bag), closed or open, containing live organism.

10.2.7.1 Wear a double layer of gloves when manipulating culture (see sections 2.6 and 10.2.9) . Prior to leaving the BSC to conduct other activities (e.g., open the incubator, record data, retrieve supplies, etc.), the outer pair of gloves must be discarded in the biohazard bin. Replace the outer gloves upon returning to the BSC.

OR

Wear a single pair of gloves when manipulating culture (see sections 2.6 and 10.2.9). Prior to leaving the BSC to conduct other activities (e.g., open the incubator, record data, retrieve supplies, etc.), discard the gloves in the biohazard bin. Put on a new pair of gloves upon returning to the BSC or before handling any vessel containing live organism.

Standard Operating Procedure (SOP) Addendum Form

OPP Microbiology Laboratory

SOP Name :	Biosafety in the Laboratory
SOP Number:	MB-01-02
Addendum Number:	A-2
Changes made by (name/date):	Michele Cottrill/3-21-01
Description of Change(s)/ Addition(s):	See attached sheet for additions (in bold text) and deletions (in strikeout text).
Justification(s) for the Change(s):	To provide additional guidance and steps on appropriate use and disposal of lab coats worn in the BSL 3 laboratory.
Approval: (Signature/Date)	
OPP Lab QA Officer	
OPP Lab Team Leader	

Date Issued: _____

Controlled Copy Number: _____

Addendum A-2/Description of Change(s)/Additions:

10.2.5 Wear disposable lab coats while working in the Biosafety Level 3 laboratory. White cloth lab coats may not be worn in the Biosafety Level 3 laboratory. Lab coats do not have to be worn while doing paperwork in the laboratory or when entering the laboratory solely to retrieve an item such as a document, *provided that no manipulation of cultures (see section 2.6) is in progress.* **See section 10.2.18 for additional guidance on disposal of lab coats.**

10.2.18 ~~Remove and discard lab coats (in biohazard bin) before exiting the Biosafety Level 3 laboratory to enter the double-door access zone.~~ No lab coats worn during manipulation (see section 2.6) of *M. bovis* BCG may be worn out of the Biosafety Level 3 laboratory. **This includes lab coats worn by personnel who are present in the laboratory but not directly involved in culture manipulation. Prior to exiting the laboratory and entering the double door access zone, remove and discard lab coats (in biohazard bin) according to the descriptions of activities provided in sections 10.2.18.1 and 10.2.18.2:**

10.2.18.1 Disposable lab coats worn while harvesting or homogenizing cultures of M. bovis BCG must be discarded in a biohazard bin before the analyst leaves the laboratory and enters the double-door access zone. The lab coat may not be stored in the laboratory for re-use later in the day.

10.2.18.2 Disposable lab coats worn for purposes other than harvesting or homogenizing cultures of M. bovis BCG during the day may be stored in a designated area in the laboratory in the event that the analyst leaves the laboratory (e.g., to retrieve an item or take a break). Upon return, the analyst may re-use the lab coat to resume laboratory activities. At the end of each day, all used disposable lab coats must be disposed of in the biohazard bin.